EVALUATION OF THE HATCHING TRIGGER AND LARVAL ECOLOGY OF THE SALAMANDER *AMPHIUMA MEANS*

Margaret S. Gunzburger[1]

Department of Biological Science, Florida State University, Tallahassee, FL 32306-1100, USA

Abstract: I investigated the early life history of the salamander *Amphiuma means*. Although adult *A. means* are entirely aquatic, eggs are often found above water levels in areas from which water has receded. A series of experiments was performed using three egg clutches of *A. means* collected from organic sediment at the bottom of a dried lake in northern Florida. The first experiment demonstrated that eggs of *A. means* hatch in response to inundation with water, and aquatic larvae completely resorbed their gills in about 2 wk. Eggs that hatched at later dates produced larger hatchlings in all three clutches and larger juveniles at metamorphosis in two clutches. A second experiment indicated that hatchlings can survive, on average, over 125 d without feeding by using resources from their yolk reserves. The third experiment showed that eggs are capable of surviving an average of 110 d (SD = 47.2 d) on moist substrate without hatching. Some eggs hatched onto the substrate without inundation, and resulting hatchlings could survive on this substrate without inundation for an average of 21 d (SD = 26.1 d). Five eggs produced hatchlings without gills during this experiment, suggesting that this species may bypass the larval period completely if eggs are not inundated. My experiments suggest that the eggs of *A. means* may be specialized for development in terrestrial nest chambers. Information on development and larval ecology of *A. means* from my study can be applied to future evaluations of the evolutionary relationships of salamander families.

Key words: *Amphiuma means*; Amphiumidae; Clutch size; Delayed hatching; Direct development; Egg; Florida; Hatchling; Larvae; Life history; Metamorphosis; Reproduction; Salamander

Amphiuma means is a large aquatic salamander found in lentic water habitats of the Coastal Plain of the southeastern United States (Petranka, 1998). Amphiumas are neotenic (i.e., retain gill slits after metamorphosis), are uniform dark brown in dorsal coloration, and have extremely reduced fore and hind limbs that result in an eel-like appearance (Salthe, 1973). Adult amphiumas are entirely aquatic, probably only rarely dispersing over land (Aresco, 2002; Snodgrass et al., 1999); prefer sluggish or standing water with much organic sediment for burrowing (Mount, 1975; Salthe, 1973); and survive drought conditions by aestivating in burrows constructed in the organic sediment of dried ponds and lakes (Knepton, 1954).

Eggs of all amphiuma species are laid in long strings connected by a rubbery outer covering (Petranka, 1998). All published accounts of *A. means* egg clutches indicate that the clutches were found in a chamber in the sediment of areas where the water had receded, sometimes under a structure such as a board or log (Brimley, 1910; Hay, 1888; Weber, 1944). The timing of egg deposition is unknown, but late stage eggs have been found in Georgia in September (Seyle, 1985), and eggs have been found in Florida in February, May, June, and July (Bancroft et al., 1983; Brimley, 1910; Weber, 1944). Oviposition in the closely related species *Amphiuma tridactylum* occurs in June in Louisiana (Fontenot, 1999). *Amphiuma means* egg clutches are always found in a chamber with an adult amphiuma. The water level at the time of initial egg deposition is unknown, so it is possible that *A. means* lay eggs in chambers in sediments beneath the shallow water edges. At least one clutch of *A. means* has been found in the nest of an American alligator, *Alligator mississippiensis* (K. Enge, personal communication), suggesting that, in some cases, *A. means* may lay eggs in moist areas above the water level.

Information on development, hatching, and larval period of *A. means* is limited. One investigator observed two eggs hatching after inundation in water overnight (Seyle, 1985). The larval period is believed to be short, although larvae of *A. means* have never been found in nature. Newly hatched larvae of *A. tridactylum* were found to resorb their gills completely within 16 d (Ultsch and Arceneaux, 1988). Larvae of *A. means* that hatched from eggs under laboratory conditions had white

[1] Correspondence: e-mail, gunz@bio.fsu.edu

gills and measured 55 mm total length (TL) (Weber, 1944). Juveniles of *A. means* without gills have been found at sizes as small as 68 mm TL (Davison, 1895).

Amphiuma means eggs may often be stranded far from current water levels during development because many lentic water habitats, including temporary ponds, river floodplains, and large lakes in the southeastern Coastal Plain, are subject to fluctuations in water levels (Myers and Ewel, 1990). It is assumed that, when stranded above the water level during times of drought, *A. means* eggs remain in the nest chamber with the female until inundation, at which point the eggs hatch and larvae can escape the chamber. However, in many situations, temporary inundation with rainwater may fill the nest chamber, but not raise the water level of the pond or lake to the level of the nest chamber. Several groups of aquatic anamniotes, including fishes (e.g., Cyprinodontiformes, DiMichele and Taylor, 1980), anurans (*Pseudophryne bibroni*, Bradford and Seymour, 1985), and caudates (several Ambystomatids, Anderson and Williamson, 1976; Marco and Blaustein, 1999; Petranka and Petranka, 1980; and *Eurycea quadridigitata*, Taylor et al., 1988), have terrestrial eggs that delay hatching until inundation (Martin, 1999). Inundation with water is the hatching trigger for some fish eggs that incubate terrestrially, such as the grunion *Leuresthes tenuis* (Griem and Martin, 2000) and the puffer *Takifugu niphobles* (Yamahira, 1997). In the case of the marbled salamander *Ambystoma opacum*, females oviposit under cover objects in dried ponds. After developing to a certain stage, eggs delay further development and hatching until rain fills the ponds and inundates the eggs (Petranka and Petranka, 1981). In this species, the reduction of oxygen availability in water is the trigger for hatching (Petranka et al., 1982).

Advantages of terrestrial eggs for aquatic organisms include an ability to reproduce in habitats with unpredictable hydroperiod and greater oxygen availability than in water (Martin, 1999). However, terrestrial eggs may have more variable survival rates and must be provided with large amounts of yolk to survive the extended incubation period (Darken et al., 1998; Martin, 1999). In order to evaluate these tradeoffs for the eggs of *A. means*, it is vital to understand both the environmental cues for hatching and the length of time larvae can survive on resources from their yolk reserves.

Information on the development and early life history of *A. means* may help to elucidate the evolutionary relationships of Amphiumidae among other salamander families. Developmental characteristics have become increasingly important to studies of evolutionary relationships among salamander groups (Wake and Marks, 1993). A recent analysis places Amphiumidae and Plethodontidae as sister groups based on morphological and molecular characters (Larson and Dimmick, 1993; but see Gao and Shubin, 2001). The common ancestor of these groups may have had a post-hatching larval period (Means, 2000). However, the most basal Plethodontids, the Desmognathinae salamanders *Phaeognathus hubrichti*, *Desmognathus aeneus*, and *D. wrighti*, have direct development (Petranka, 1998; Tilley and Mahoney, 1996; Titus and Larson, 1996). More derived Desmognathinae salamanders have a larval period, as do the most basal Plethodontinae salamanders, the Hemidactyliini (Lombard and Wake, 1986; Petranka, 1998). Direct development arises again in the Plethodontinae in tribes Plethodontini and Bolitoglossini (Wake and Marks, 1993). Information on the developmental characteristics of *A. means* may increase the confidence of the sister grouping of Amphiumidae and Plethodontidae and provide insight into the likely development pattern of the common ancestor of these groups.

The goal of my study was to evaluate the hatching trigger and larval ecology of *A. means* to gain insight into the possibility that eggs of this species may be specialized for development in terrestrial chambers. In addition, the new information I provide on the development of *A. means* can be applied to future studies of the evolutionary relationships of salamander families. Experiments were designed with the following specific objectives: (1) to determine if the eggs of *A. means* are triggered to hatch by inundation with water, (2) to evaluate the relationship of hatching date (egg age) with size at hatching and transformation, (3) to evaluate the length of the larval period, (4) to determine how long juveniles can survive on yolk reserves, and (5) to determine how long eggs can remain viable without hatching.

TABLE 1.—Descriptions of egg clutches and attendant adult of *Amphiuma means* collected at Lake Iamonia, Leon County, Florida. Clutch weight was estimated for each clutch using the weight of 20 eggs from Clutch A. Egg size is diameter in mm of the outer egg capsule; attendant adult SVL and TL are in mm. Clutch B was collected with an adult, but this adult was not measured; this is the largest reported post-oviposition clutch size for *A. means* (but see Rose [1966] for larger clutch sizes based on enlarged ova). Clutch C probably represents a partial clutch of eggs.

	Clutch			Attendant adult			
	Date collected	# Eggs	Weight (g)	Egg size	SVL	TL	Weight (g)
A	26 July 2000	143	129	8.2	550	715	350
B	27 July 2000	210	189	10.1	—	—	—
C	2 August 2000	87	78	9.8	610	805	510

METHODS

Three egg clutches of *A. means* were collected from Lake Iamonia, Leon County, Florida during July and August 2000 (30° 38.528′ N, 84° 12.139′ W; Table 1). Lake Iamonia is a 2330-ha sinkhole lake that was almost completely dry by December 1999 due to a prolonged drought in northern Florida. Clutches were uncovered through the work of machinery (large backhoe) being used to remove organic sediment from the lake bottom. Eggs and hatchlings were maintained at the Florida State University Biological Research Facility Greenhouse in Tallahassee, Leon County, Florida, where my experiments were conducted.

General care of eggs was as follows. Prior to the beginning of experiments, each egg clutch was divided into two sections using natural separations among egg strands and maintained in round lidded plastic containers (16 cm diameter, 11 cm deep) lined with unbleached paper towels moistened with well water (pH = 7.1). Egg clutches were separated into groups for the three experiments on 22 August 2000 for Clutch B and 19 September 2000 for Clutches A and C. An attempt was made to reduce the number of times the string between eggs was cut by using pre-existing separations among the eggs. However, in order to separate the eggs, the strand connecting the eggs was cut several times for each clutch. Clutches A and C were divided into groups of five eggs, and Clutch B was divided into groups of 10 eggs. This resulted in 14 containers for Clutch A, 19 containers for Clutch B, and 10 containers for Clutch C (Table 2). Of these, most containers were inundated on a schedule for Experiment 1. However, one container from Clutches A and C and three containers from Clutch B were used for Experiment 3.

During the experiments, each group of eggs was maintained on moistened paper towels in a tightly lidded, square, semi-opaque white, plastic container (10 × 11 × 4 cm, Republic brand "Freezette" container) with holes drilled in the lid for ventilation. Paper towels and water were changed as necessary, at least every 4 d throughout the experiment. Containers were exposed to indirect ambient light conditions but were shaded to reduce light intensity and temperature. Temperature in the greenhouse ranged 15–30 C throughout the 8-mo period of these experiments.

Experiment 1: Inundation of Eggs

The objectives of this experiment were: (1) to determine if inundation with water triggers eggs to hatch, (2) to determine if hatching date

TABLE 2.—Inundation schedule for *Amphiuma means* containers for Experiment 1. Single-digit container numbers had an intact string of five (Clutches A and C) or 10 (Clutch B) eggs, while double-digit containers contained an equal number of eggs connected in shorter strings (see Methods for details). Day = day of inundation from beginning of experiment, Date = actual date of inundation. * indicates containers reserved for Experiment 2 that examined hatchling longevity.

Clutch B	Inundation		Clutch A	Clutch C	Inundation	
Container	Day	Date	Container	Container	Day	Date
1*, 11	1	22 August 2000	1*	1*	1	19 September 2000
7, 17*	6	27 August 2000	6*, 16	2*, 12	6	24 September 2000
4*, 14	11	1 September 2000	3, 13*	15*, 16	13	1 October 2000
3, 13	16	6 September 2000	17, 18*	3, 13	21	9 October 2000
8, 18	21	11 September 2000	2, 12	4, 14	26	14 October 2000
2, 12	26	16 September 2000	5, 15	—	31	21 October 2000
5, 15	31	21 September 2000	4, 14	—	40	30 October 2000
6, 16	36	26 September 2000				

TABLE 3.—Comparison of TL at hatching and transformation for three clutches of *Amphiuma means* eggs over three hatching intervals from Experiment 1. The TL values are means in mm with standard deviations in parentheses; n = number of eggs from each clutch in that hatching interval. ANOVA *P*-values are listed for each comparison; an * indicates a significant difference. A Bonferroni post-hoc test was used to determine which values were significantly different for the comparison of TL at hatching for hatch days 16–30; values with the same letter are not significantly different ($P > 0.05$).

Hatch days	Clutch	n	Hatching		Transformation	
			TL	P	TL	P
1–15	A	24	57 (1.7)		65 (2.6)	
	B	65	57 (3.9)	0.61	64 (4.3)	0.52
	C	24	57 (1.8)		67 (2.5)	
16–30	A	20	60 (2.8) a		67 (2.5)	
	B	64	62 (3.4) b	0.001*	68 (2.9)	0.53
	C	19	58 (2.3) a		67 (1.8)	
31–45	A	19	61 (2.2)		66 (2.3)	
	B	14	64 (3.9)	0.01*	70 (3.6)	0.001*

is correlated with size at hatching and transformation, and (3) to determine the length of the larval period. To standardize the clutches for differences in stage of development, experimental treatments were started for each clutch when one egg appeared to begin hatching (indicated by a bulge protruding from the egg capsule). At this point, each clutch was separated into groups and the experimental treatments began. Clutch B was at a later stage of development than Clutches A and C when collected and, hence, began this experiment at an earlier date (Table 2). Throughout this experiment, dead eggs were identified by the presence of fungus or bacterial growth and were removed from the containers.

The effect of severing the connection between eggs was unknown, so, for each clutch, a container with an intact string of eggs (i.e., 10 eggs connected for Clutch B) was paired with a container with the same number of eggs not connected in one string (i.e., strings of three, three, and four eggs to result in 10 total eggs for Clutch B). An inundation schedule of two containers every 5–7 d was constructed by randomly assigning pairs of containers to each inundation date (Table 2). This design allowed me to assess the effects of egg age and inundation separately: if eggs hatched once a specific development stage was reached, then all eggs would be expected to hatch at the same time regardless of inundation schedule. How-

ever, if inundation is required for eggs to hatch, then eggs would be expected to postpone hatching until their container was filled with water. Inundated containers were filled with approximately 180 ml of well water, and a folded paper towel was placed in the container as a hiding place for larvae. The effect of inundation on proportion of eggs hatching was evaluated using a paired *t*-test. For each clutch, the proportion that hatched after inundation was compared to the expected proportion if inundation has no effect on hatching (0.05). A significant result in this test indicates that inundation affects the proportion hatching.

Upon hatching, total length (TL) of all hatchlings was measured by briefly immobilizing each hatchling in a zip-top plastic bag with a small amount of water and measuring to the nearest mm with a ruler. Because measuring hatchlings was difficult and to reduce stress to the animals, TL was used instead of snout–vent length (SVL) because the TL measurement could be taken much faster. Because there was no possibility of tail injury to hatchlings prior to measurement, there was no potential for bias in TL to SVL ratios by possible tail regrowth, as could be problematic in wild-caught individuals. In order to estimate the amount of yolk remaining at hatching, the length of yolk visible through the ventral body wall in each hatchling was also measured using a ruler. Gills of hatchlings were typically pink with circulating blood. I recorded the number of days that the gills remained pink before turning gray, at which time, presumably, they were no longer used for respiration. Then, I recorded TL at transformation (i.e., when the gills were absorbed to tiny nubs).

The data from all three clutches in Experiment 1 could be pooled for analysis if the three clutches were the same age and hatched at similar sizes. In order to determine if data from the three clutches could be pooled for analysis of length measurements, TL of hatchlings at hatching and transformation was compared among the three clutches using analysis of variance (ANOVA). Three hatching intervals were compared: hatch dates 1–15, 16–30, and 31–45 d. This analysis indicated that hatchling size differed significantly among the clutches for three of these six comparisons (Table 3). In addition, because the age of these

egg clutches (i.e., date of oviposition) was not known, hatching date for each clutch relative to actual egg age was unknown. I attempted to control for variation in stage of development by starting the experiment at a similar developmental stage for each clutch, but the actual ages of the eggs may have been very different at the start of the experiment if development rate was variable. Thus, because size at hatching and transformation were found to differ from some comparisons and the actual age of each clutch was unknown, size at hatching and transformation was analyzed separately for each clutch. I used a linear regression analysis to examine the relationship between size at hatching and transformation and hatching date for hatchlings from each clutch.

Experiment 1 ended when all hatchlings in the last containers inundated for each clutch had transformed. Several containers of juveniles were maintained for Experiment 2. The remaining juveniles were released at Lake Iamonia in shallow water near the site of collection.

Experiment 2: Hatchling Longevity

The objective of Experiment 2 was to determine how long juveniles can survive on yolk reserves and was accomplished by continuing to maintain some containers of hatchlings from Experiment 1. Four containers from Clutch A (19 juveniles), three containers from Clutch B (30 juveniles), and three containers from Clutch C (14 juveniles) from Experiment 1 were selected to be used for Experiment 2 (Table 2). These containers were generally inundated early in Experiment 1, so they represented the oldest juveniles from each clutch. Juvenile survival was measured as number of days from hatching that each juvenile survived.

Experiment 3: Egg Longevity

The objective of Experiment 3 was to determine how long eggs could survive on the moist paper towels without inundation. This experiment began the same day as Experiment 1 for each clutch. Eggs for this experiment were maintained under identical conditions as those in Experiment 1, but were never inundated. This experiment consisted of three containers of 10 eggs from Clutch B, one

container of five eggs from Clutch A, and one container of seven eggs from Clutch C. Eggs were monitored daily and the fate of each egg was recorded; dead eggs and hatchlings were removed. Hatchlings were measured at the time of hatching as in Experiment 1. I used an ANOVA to compare the average TL of hatchlings from all containers in Experiment 3 with the average TL of all hatchlings from Experiment 1, pooled over all three clutches. Data were pooled for this analysis because there were too few hatchlings from each clutch in Experiment 3 for separate analysis. All statistical analyses were performed using SYSTAT 9.0 (SPSS Inc., 1999).

RESULTS

Each egg clutch was found with an adult *A. means* and was collected from an area of lake bottom that had been dry for several months and was approximately 100 m from the closest remaining water (Table 1). Sedges (*Cyperus* sp.), dog fennel (*Eupatorium compositifolium*), and other vegetation had grown over the dried soil, and clutches were found about 50 cm below the surface in a moist peaty layer of soil. Embryos were clearly visible within the egg capsules; all embryos had well developed legs at the time of collection, and gills with circulating blood were visible. Clutch B had darkly pigmented embryos, while Clutches A and C had lighter, earlier stage embryos. Embryos were observed actively moving inside the eggs, and, if the eggs were disturbed, the embryos tended to return to a position within the egg capsule in which the developing embryo was positioned above the remaining yolk.

Experiment 1: Inundation of Eggs

Inundation with water triggered hatching of eggs of *A. means* within 24–48 h for up to 40 d following apparent readiness to hatch. Throughout Experiment 1, for all three clutches pooled, 16 eggs hatched prior to inundation, while 242 hatched after inundation ($t = 13.81$, df $= 2$, $P = 0.005$; Table 4). Several eggs observed after inundation hatched within 3 h. Most eggs hatched within 24 h of inundation, but 1 egg from Clutch C and 10 eggs from Clutch A (from five different containers) hatched within 48 h after inunda-

TABLE 4.—Summary of hatchling characteristics for three clutches of *Amphiuma means* eggs used in Experiment 1, pooled over all containers for each clutch. Means are followed by standard deviations in parentheses; inun = inundation with water; yolk length = length of yolk visible through ventral surface at hatching.

| | # Hatchlings | | TL (mm) at | | | |
Clutch	Before inun	After inun	Hatching	Transformation	Yolk length	Larval period (d)
A	0	67	59 (2.6)	66 (2.6)	23 (2.0)	14 (2.1)
B	15	132	60 (4.5)	66 (4.3)	23 (1.8)	14 (2.5)
C	1	43	57 (2.1)	65 (2.5)	24 (1.4)	16 (1.3)

tion. Of the 16 individuals that hatched onto the paper towels prior to inundation, 13 survived until their container was inundated, with survival times ranging from 1–31 d ($\bar{x}$ = 3.4 ± SD 11.7 d) on the paper towels. These hatchlings survived after their containers were inundated until the end of the experiment. The other three hatchlings did not survive until inundation and survived an average of 4.5 d (SD = 2.1), ranging from 3–6 d. Eggs remained viable throughout the experiment; a total of six embryos from all three clutches died during this experiment; five additional embryos died while in the process of hatching prior to inundation. Severing the string between eggs had no effect on egg survival or hatching rate as almost all eggs hatched in each container.

Linear regression indicated that size at hatching is positively related to hatching date in all clutches, with later hatchlings emerging at larger sizes (Fig. 1). Hatchlings of all three clutches had similar amounts of yolk remaining at hatching, and yolk length was about one-third the total body length of hatchlings (Table 4). All hatchlings in this experiment hatched with gills. Hatchlings were observed to gulp air immediately after hatching, and gills typically turned gray 3–7 d after hatching. The length of larval period was similar for the three clutches, averaging 14.5 d (SD = 2.3) (Table 4). Usually all hatchlings in a container that hatched on the same day also transformed on the same day. Size at transformation was not constant throughout the experiment, but was positively correlated with date of hatching for Clutches B and C, but not Clutch A (Fig. 2). Direct relationships between size at hatching and size at transformation could not

be evaluated because individual hatchlings were not marked.

Four *A. means* hatched with deformities during Experiment 1. One hatchling each from Clutch A and Clutch B appeared to have crooked bodies, possibly a spinal deformity. One hatchling from Clutch B was attached in a coil to its yolk and was unable to straighten itself. Another hatchling from Clutch B had an extreme constriction of the body just anterior to the cloaca. All hatchlings with deformities hatched alive but were poor swimmers and were noticeably less active than their normal siblings.

Experiment 2: Hatchling Longevity

After hatching, juveniles of *A. means* were able to survive without feeding for several months in water. Average juvenile survival was 125.6 d (SD = 7.0) for Clutch A , 154 d (SD = 7.7) for Clutch B, and 144 d (SD = 5.2) for Clutch C. The maximum juvenile survival after hatching without feeding was 175 d.

Experiment 3: Egg Longevity

The containers maintained with moistened paper towels to evaluate egg longevity had a total of 42 eggs from the three clutches (Fig. 3). Of these, 19 eggs died without hatching after an average of 110.1 d (SD = 47.2), ranging 31–165 d. Three hatchlings died while in the process of hatching; apparently they were unable to fully escape from the egg capsule. Twenty eggs hatched successfully on an average hatching date of 93.3 d (SD = 45) after the beginning of the experiment, ranging 5–149 d (Fig. 3). Average size of hatchlings (TL $\bar{x}$ = 62.2 ± SD 5.5) in this experiment was larger than the overall average size of hatchlings from Experiment 1 (ANOVA, F = 7.765, P = 0.006). Average survival after hatching was 21.1 d (SD = 26.1), ranging from 1–91 d. The last five hatchlings (all from Clutch B) in this experiment hatched with gills fully adsorbed at an average hatch date of 139.6 d (SD = 13.9), over 100 d after the last hatchlings from Clutch B in Experiment 1 and 18 d after the last hatchlings with gills from Clutch B in this experiment. These hatchlings had an average TL of 65.8 mm (SD = 4.9).
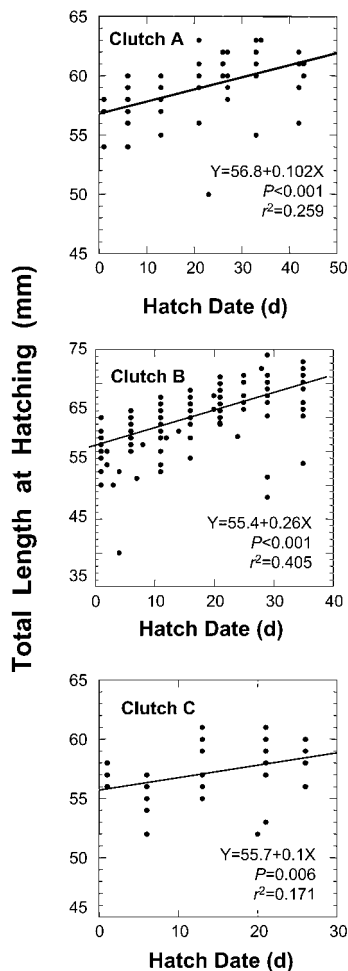
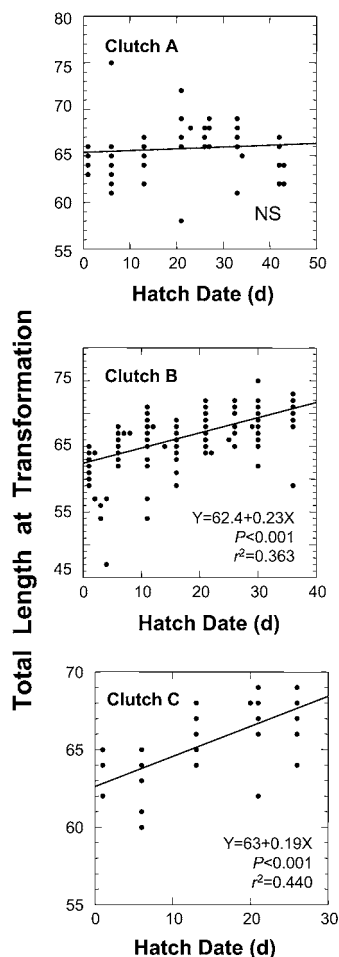FIG. 1.—Regression of size at hatching (TL in mm) against hatch date for three clutches of *Amphiuma means*.

FIG. 2.—Regression of size at transformation (TL in mm) against hatch date for three clutches of *Amphiuma means*.

DISCUSSION

The results of my study indicate that the development of the eggs and larvae of *A. means* is plastic and may be strongly influenced by environmental characteristics. The experiments demonstrated that the eggs of *A. means* are capable of delayed hatching and that hatching is triggered by inundation with water, similar to other aquatic species with terrestrial eggs (Griem and Martin, 2000; Petranka et al., 1982; Yamahira, 1997). However, eggs that delay hatching due to later inundation suffer from increased mortality. In my study, the *A. means* eggs held without inundation (Experiment 3) suffered a 50% mortality rate prior to hatching, while eggs that were inundated (Experiment 1) had very low mortality (4%). A similar pattern was found for the grunion *L. tenuis*, where eggs that delayed hatching had decreased survival and decreased hatching success over time (Darken et al., 1998). Thus, in nature, *A. means* eggs inundated early in development may have increased hatching rate than eggs that wait several months prior to inundation.

Eggs that are inundated later in development may produce larger hatchlings and juveniles at transformation. In my study, eggs that hatched at later dates produced larger hatchlings in all clutches and larger metamorphs in two of the three clutches (Figs. 1, 2). Delayed hatching of the salamander *Ambys-*
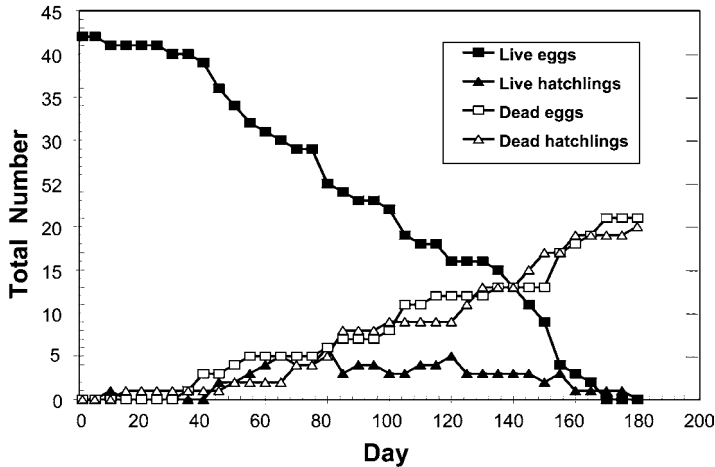
Fig. 3.—Cumulative number of eggs and hatchling/juvenile *Amphiuma means* alive and dead over time, for eggs never inundated with water (Experiment 3). Totals are for the three clutches pooled, but clutches began this experiment on different dates. Day = day from beginning of experiment for each clutch. All individuals from Clutch C had died by day 84; all individuals from Clutch A had died by day 158; thus, all remaining individuals were from Clutch B.

toma barbouri resulted in larger hatchlings that were more likely to escape predation (Sih and Moore, 1993). If hatching at a larger size is beneficial, then selection would favor a longer developmental period for eggs of *A. means*, regardless of timing of inundation, in order to produce larger hatchlings. However, perhaps once a certain development stage is passed, the eggs will hatch if inundated due to physiological or other constraints. Unfortunately, with a limited number of eggs available for my research, I could not investigate the effect of early inundation on egg development, development rate, and hatching date. The length of the larval period of *A. means* appeared relatively constant for all clutches in Experiment 1, so later hatchlings were also larger at transformation. Larger size at metamorphosis has also been shown to increase survival and fitness in other salamander species (Scott, 1994).

Adults of *A. means* appear to face a trade-off when selecting oviposition sites. If a site is inundated late relative to egg development, eggs will hatch later and produce larger hatchlings, but may suffer greater mortality. In contrast, a site that is inundated early relative to egg development will result in eggs that hatch sooner and have a higher rate of hatching, but produce smaller offspring. The degree to which adults of *A. means* can influence the timing of inundation of their eggs through

selection of oviposition sites is completely unknown, although there is some evidence that *Ambystoma opacum* has such a mechanism (Petranka and Petranka, 1981). Possible mechanisms by which adult *A. means* may influence hatching date could involve ovipositing at certain distances above the current water level or optimally timing oviposition in anticipation of inundation events. More data from censuses of oviposition sites in nature may provide insight into these possibilities. However, because there probably exists a collection bias toward areas where water levels have receded, controlled experiments evaluating oviposition site selection may be necessary.

The majority of juveniles in my study that hatched in water survived over 120 d without feeding, providing evidence that hatchlings in nature may be capable of overwintering in the nest chamber if water levels remain low. In addition, the ability of juveniles to survive on the moist paper towels suggest that, if eggs are inundated and hatch but then water levels recede, juveniles may still be able to survive provided the moisture level in their nest chamber is high. A similar developmental characteristic has been found for the frog *P. bibroni* whose terrestrial eggs are triggered to hatch by inundation. However, larvae are sometimes left stranded by receding water levels (Geiser and Seymour, 1989). Larval *P. bibroni* can survive on moist substrates with-

out inundation for up to 100 d; experiments demonstrated that survival is significantly increased by higher water potential of the substrate and lower temperatures (Geiser and Seymour, 1989). Larvae of *A. means* that hatch terrestrially may reduce water loss by rapidly absorbing their gills; gills may not be critical to respiration because blood circulation though the gills usually ceased by several days after hatching in water. In the terrestrially developing frog *Agalychnis callidryas*, the rate of post-hatching gill absorption increases with increasing embryo age at hatching and oxygen availability in water (Warkentin, 2000). In my study, late hatching *A. means* had similar larval periods (i.e., gill absorption rates) as early hatchlings under similar environmental conditions. However, future research on hatchlings of *A. means* may show that this species also has the ability to regulate gill absorption rate in response to environmental conditions.

My research suggests that the eggs of *A. means* may be specialized for development in terrestrial nest chambers. I found that hatching can be triggered by inundation, survival of hatchlings is extended by using yolk reserves, and the larval period can be bypassed completely. Additional characteristics that probably contribute to the ability of *A. means* eggs to survive terrestrially are a rubbery outer covering of the eggs and large amounts of yolk reserves in the eggs. In addition, attendance of eggs by adult *A. means* may increase survival of eggs in terrestrial nest chambers, as in other species of salamanders with parental brooding behavior (Dennis, 1962; Tilley, 1972).

In summary, my results indicate that the eggs and hatchlings of *A. means* clearly are adapted to survive in environments with fluctuating water levels. The eggs of *A. means* can probably resist desiccation, as well as the eggs of many terrestrial plethodontids, due to their rubbery outer covering. The conditions under which some of the eggs of *A. means* hatched in my study were similar to those used to successfully hatch the eggs of the plethodontid salamanders *Hemidactylium scutatum* and *D. ochropheus* (Houck et al., 1985; Vess and Harris, 1997). In addition, eggs of *A. means* are capable of hatching and surviving with moisture levels that are probably similar to that of directly developing plethodontids,

although *A. means* larvae usually hatch with gills. My research suggests the possibility that the common ancestor of the Amphiumidae and Plethodontidae had a very short larval period, or perhaps showed extreme plasticity in the timing of developmental events.

LITERATURE CITED

ANDERSON, J. D., AND G. K. WILLIAMSON. 1976. Terrestrial mode of reproduction in *Ambystoma cingulatum*. Herpetologica 32:214–221.

ARESCO, M. J. 2002. *Amphiuma means* (two-toed Amphiuma) overland migration. Herpetological Review 33:296–297.

BANCROFT, J. S., A. GODLEY, B. GROSS, C. ROJAS, AND D. SUTPHEN. 1983. Large-scale operations management test of the use of the white amur for control of problem aquatic plants: the herpetofauna of Lake Conway: species accounts. U.S. Army Corps of Engineers Miscellaneous Paper A–83–5.

BRADFORD, D. F., AND R. S. SEYMOUR. 1985. Energy conservation during the delayed-hatching period in the frog *Pseudophryne bibroni*. Physiological Zoology 61:470–474.

BRIMLEY, C. S. 1910. Records of some reptiles and batrachians from the southeastern United States. Proceedings of the Biological Society of Washington 23:9–18.

DARKEN, R. S., K. L. M. MARTIN, AND M. C. FISHER. 1998. Metabolism during delayed hatching in terrestrial eggs of a marine fish, the grunion *Leuresthes tenuis*. Physiological Zoology 71:400–406.

DAVISON, A. 1895. A contribution to the anatomy and phylogeny of *Amphiuma means*. Journal of Morphology 11:375–410.

DENNIS, D. M. 1962. Notes on the nesting habits of *Desmognathus fuscus* (Raf.) in Licking County, Ohio. Journal of the Ohio Herpetogixcal Society 3:28–35.

DIMICHELE, L., AND M. H. TAYLOR. 1980. The environmental control of hatching in *Fundulus heteroclitus*. Journal of Experimental Zoology 214:181–187.

FONTENOT, C. L., JR. 1999. Reproductive biology of the aquatic salamander *Amphiuma tridactylum* in Louisiana. Journal of Herpetology 33:100–105.

GAO, K., AND N. H. SHUBIN. 2001. Late Jurassic salamanders form Northern China. Nature 410:574–577.

GEISER, F., AND R. S. SEYMOUR. 1989. Influence of temperature and water potential on survival of hatched, terrestrial larvae of the frog *Pseudophryne bibroni*. Copeia 1989:207–209.

GRIEM, J. N., AND K. L. M. MARTIN. 2000. Wave action: the environmental trigger for hatching in the California grunion *Leuresthes tenuis* (Teleostei: Atherinopsidae). Marine Biology 137:177–181.

HAY, O. P. 1888. Observations on Amphiuma and its young. American Naturalist 22:315–321.

HOUCK, L. D., S. G. TILLEY, AND S. J. ARNOLD. 1985. Sperm competition in a Plethodontid salamander: preliminary results. Journal of Herpetology 19:420–423.

KNEPTON, J. C., JR. 1954. A note on the burrowing habits of the salamander *Amphiuma means means*. Copeia 1954:68.

LARSON, A., AND W. W. DIMMICK. 1993. Phylogenetic relationships of the salamander families: an analysis of congruence among morphological and molecular characters. Herpetological Monographs 7:77–93.

LOMBARD, R. E., AND D. B. WAKE. 1986. Tongue evolution in the lungless salamanders, family Plethodontidae. IV. Phylogeny of Plethodontid salamanders and the evolution of feeding dynamics. Systematic Zoology 35:532–551.

MARCO, A., AND A. R. BLAUSTEIN. 1999. Egg gelatinous matrix protects *Ambystoma gracile* embryos from prolonged exposure to air. Herpetological Journal 8:207–211.

MARTIN, K. L. M. 1999. Ready and waiting: delayed hatching and extended incubation of anamniotic vertebrate terrestrial eggs. American Zoologist 39:279–288.

MEANS, D. B. 2000. Southeastern U.S. coastal plain habitats of the Plethodontidae: the importance of relief, ravines, and seepage. Pp. 287–302. *In* R. C. Bruce, R. J. Jaeger, and L. D. Houck (Eds.), The Biology of Plethodontid Salamanders. Kluwer Academic/Plenum Publishers, New York, New York, U.S.A.

MOUNT, R. H. 1975. The Reptiles and Amphibians of Alabama. Auburn Printing Company, Auburn, Alabama, U.S.A.

MYERS, R. J., AND J. J. EWEL. 1990. Ecosystems of Florida. University of Central Florida Press, Orlando, Florida, U.S.A.

PETRANKA, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C., U.S.A.

PETRANKA, J. W., AND J. G. PETRANKA. 1980. Selected aspects of the larval ecology of the marbled salamander *Ambystoma opacum* in the southern portion of its range. American Midland Naturalist 104:352–36.

———. 1981. On the evolution of nest site selection in the marbled salamanders, *Ambystoma opacum*. Copeia 1981:387–391.

PETRANKA, J. W., J. J. JUST, AND E. C. CRAWFORD. 1982. Hatching of amphibian eggs: the physiological trigger. Science 217:257–259.

ROSE, F. L. 1966. Reproductive potential of *Amphiuma means*. Copeia 1966:598–599.

SALTHE, S. N. 1973. *Amphiuma means* garden, two-toed congo eel. Catalogue of American Amphibians and Reptiles 148:141–142.

SCOTT, D. E. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. Ecology 75:1383–1396.

SEYLE, C. W., JR. 1985. *Amphiuma means* reproduction. Herpetological Review 16:51–52.

SIH, A., AND R. D. MOORE. 1993. Delayed hatching of salamander larvae in response to enhanced larval predation risk. American Naturalist 142:947–960.

SNODGRASS, J. W., J. W. ACKERMAN, A. L. BRYAN, JR., AND J. BURGER. 1999. Influence of hydroperiod, isolation, and heterospecifics on the distribution of aquatic salamanders (*Siren* and *Amphiuma*) among depression wetlands. Copeia 1999:107–113.

SPSS INC. 1999. SYSTAT 9. Chicago, Illinois, U.S.A.

TAYLOR, B. E., R. A. ESTES, J. H. K. PECHMANN, AND R. D. SEMLITSCH. 1988. Trophic relationships in a temporary pond: larval salamanders and their microinvertebrate prey. Canadian Journal of Zoology 66:2191–2198.

TILLEY, S. G. 1972. Aspects of parental care and embryonic development in *Desmognathus ochrophaeus*. Copeia 1972:532–540.

TILLEY, S. G., AND M. J. MAHONEY. 1996. Patterns of genetic differentiation in salamanders of the *Desmognathus ochrophaeus* complex (Amphibia: Plethodontidae). Herpetological Monographs 10:1–42.

TITUS, T. A., AND A. LARSON. 1996. Molecular phylogenetics of Desmognathine salamanders (Caudata: Plethodontidae): a reevaluation of evolution in ecology, life history, and morphology. Systematic Biology 45:451–472.

ULTSCH, G. R., AND S. J. ARCENEAUX. 1988. Gill loss in larval *Amphiuma tridactylum*. Journal of Herpetology 22:347–348.

VESS, T. J., AND R. N. HARRIS. 1997. Artificial brooding of salamander eggs. Herpetological Review 28:80.

WAKE, D. B., AND S. B. MARKS. 1993. Development and evolution of Plethodontid salamanders: a review of prior studies and a prospectus for future research. Herpetologica 49:194–203.

WARKENTIN, K. M. 2000. Environmental and developmental effects on external gill loss in the red-eyed treefrog, *Agalychnis callidryas*. Physiological and Biological Zoology 73:557–565.

WEBER, J. A. 1944. Observations on the life history of *Amphiuma means*. Copeia 1944:61–62.

YAMAHIRA, K. 1997. Hatching success affects the timing of spawning by the intertidally spawning puffer *Takifugu niphobles*. Marine Ecology Progress Series 155:239–248.